Minireview

Protein biosynthesis: structural studies of the elongation cycle

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Abstract The elongation cycle of protein synthesis on ribosomes is catalyzed by the elongation factors EF-Tu and EF-G. A thorough crystallographic analysis of the structures of the different functional states of EF-Tu has been made. Furthermore, the structure of EF-G:GDP is the form of EF-G that dissociates from the ribosome. Since it mimics the structure of the ternary complex of EF-Tu:GTP with aminoacyl-tRNA, which subsequently binds to the ribosome, EF-G:GDP leaves an imprint on the ribosome for the ternary complex. In addition, electron cryomicroscopy studies of ribosomes with tRNA as well as the ternary complex bound are beginning to give a solid structural basis for the functional description of elongation.

Key words: Protein synthesis; Structural analysis; Elongation factor; Crystallography; Electron cryomicroscopy

1. Introduction

The transformation of genetic information on DNA into functional proteins in a living cell is a complicated process involving many RNA and protein molecules. One part is the translation of the transcribed information on mRNA on the ribosome which is a large complex of ribosomal RNA and protein. Protein synthesis is catalyzed by protein factors, some of which are GTPases (G-proteins). Translation is divided into three phases: initiation, elongation and termination. Extensive structural information has been obtained for the elongation factors. Much structural information has also been obtained for ribosomal proteins and fragments of ribosomal RNA [1]. Structural information about complete ribosomes in different functional states has been obtained by electron cryomicroscopy (cryo-EM) [2–5] and for ribosomal subunits by X-ray crystallography [6].

This review is based on the structural information about the elongation factors shedding light on the functional steps in the elongation cycle of protein biosynthesis. We will mostly concentrate on a few relevant points arising from the structural information.

2. The ribosome

Cryo-EM has provided a new level of structural acquaintance with the bacterial ribosome at resolutions better than 20 Å [2–4]. The structural studies of the ribosomal subunits have identified channels and structural details which yet remain somewhat anonymous, but the localization of proteins and rRNA is in progress [7–9]. The A, P, and E binding sites for the tRNAs have been located [2,3] and the binding of elongation factors is in progress [4]. The contacts of the tRNAs with the 30S subunit have given a clear identification of the decoding site while the acceptor end of the tRNA has helped to locate the peptidyl transfer site [2,3]. The analysis of the binding of elongation factor Tu (EF-Tu) to the ribosome [4] has furthermore resolved an old conflict concerning the position on the ribosome of the aminoacylated CCA end of the tRNA when bound to EF-Tu. It is evident that the classical factor binding site below the L7/L12 stalk on the large subunit is the factor binding site and that the acceptor end of the tRNA is associated with the factor as discussed below. Thus, as long as EF-Tu binds to the ribosome the aminoacyl part of the tRNA is nowhere near the peptidyl transfer site. The binding of elongation factor G (EF-G) will be the next challenge for cryo-EM. Since the factors, like all G-proteins, go through conformational changes in their functional cycle (see below) it can be expected that they display several conformations on the ribosome.

3. Elongation factor Tu

EF-Tu:GTP protects the aminoacyl-tRNA (aa-tRNA) against hydrolysis, and assists the ribosome in making a correct interaction between the current codon on the mRNA in the ribosomal A site and the anticodon of aa-tRNA [10]. Upon such a correct interaction the ribosome stimulates the GTP hydrolysis of EF-Tu, after which EF-Tu:GDP is released from the ribosome. The antibiotic kirromycin will inhibit this release mechanism. EF-Tu:GDP is recycled into its active form EF-Tu:GTP by the nucleotide exchange factor, EF-Ts.

All functionally relevant complexes of EF-Tu have been studied. Thus the structures of EF-Tu:GDPNP from both *Thermus thermophilus* [11] and *T. aquaticus* [12] have been determined. GDPNP is a non-hydrolyzable analogue of GTP. Recently, the structures of intact EF-Tu:GDP from *T. aquaticus* [13] and *Escherichia coli* [13,14] have been published. EF-Tu functions as a molecular switch by altering the relative orientation of the nucleotide binding domain 1 (or G-domain) to domains 2 and 3 which are held together by strong interactions [11]. Thus the activation or inactivation by recharging EF-Tu with GTP or by hydrolyzing the GTP is accompanied by very large conformational changes of these two parts relative to each other [11,12].

The nucleotide exchange achieved by the complex of EF-Tu:EF-Ts is apparently performed by physically separating the two functional parts of EF-Tu as has been observed in

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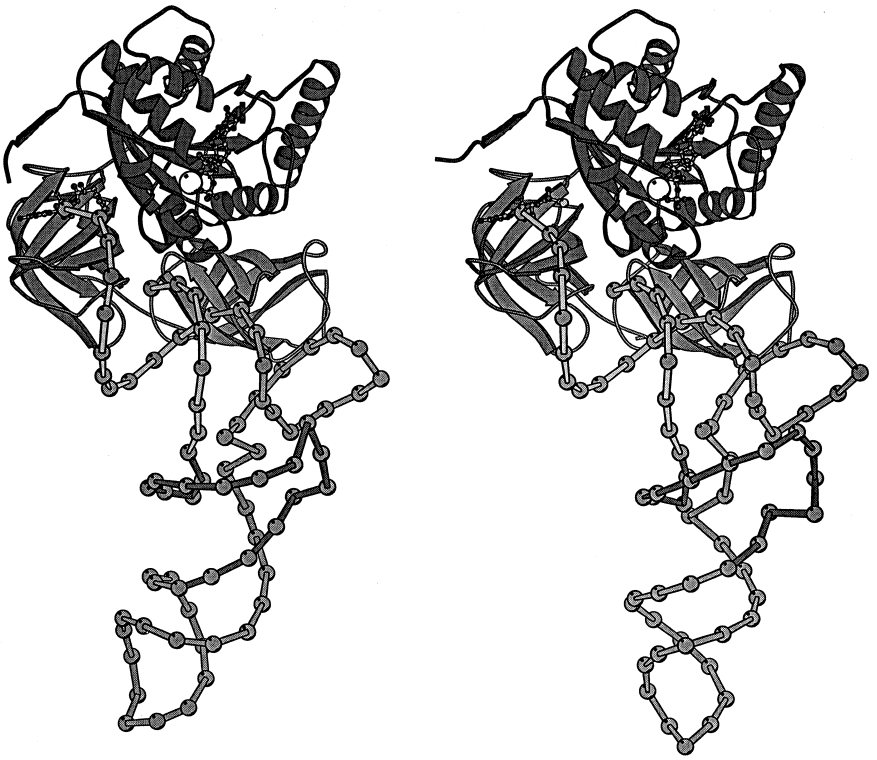

Fig. 1. Comparison of ternary complexes of EF-Tu with Phe-tRNA and Cys-tRNA. To the left is shown the complex of yeast Phe-tRNA and *T. aquaticus* EF-Tu:GDPNP. To the right is the structure of the complex of *E. coli* Cys-tRNA and *T. aquaticus* EF-Tu:GDPNP. The domains of EF-Tu are shown in cartoons in shadings of dark gray to light gray from the N- to the C-terminal, while the tRNAs are shown in backbone tracings only. Notice that there are local variations of the structure of Cys-tRNA as compared to Phe-tRNA. Notice also that the anticodon helix of Cys-tRNA makes a larger angle with the rest of the tRNA. The picture is drawn with MOLSCRIPT [42].

structures of the proteins from *E. coli* ([15]; S. Thirup, personal communication) and *T. thermophilus* [16]. These two structures raise as many questions as answers are given.

The structure of the monomer of EF-Ts from *E. coli* [15] has an internal pseudo-two-fold symmetry, which is reflected in the sequence. The N-terminal part of EF-Ts interacts with domain 1 of EF-Tu, while the C-terminal part interacts with domain 3. The C-terminal part has a protruding helix hairpin, which forms part of the interface to another pseudo-symmetric monomer. The resulting dimer of EF-Ts (consisting of four pseudo-related units) thus has two binding sites for EF-Tu on the same surface of the dimer. The structure of the fully symmetric dimer of EF-Ts from *T. thermophilus* [16] has a structure which is similar to the pseudo-symmetric monomer of EF-Ts from *E. coli*. In fact the monomer from *T. thermophilus* resembles most the C-terminal part of EF-Ts from *E. coli*, including the protruding helix hairpin. The symmetric dimer (consisting of two identical units) therefore also exposes two identical binding sites for EF-Tu, but now on opposite surfaces. Although the overall structures of the two complexes are very different, both structures can be said to have a stoichiometry of EF-Tu:$(EF\text{-}Ts)_2$:EF-Tu.

Whether these structures really represent functional states is at the moment hard to tell, but if they do not, what is then the function of the protruding helix hairpin? Although the structures show how EF-Ts modifies the local structures of many of the nucleotide binding loops, the detailed mechanism of nucleotide exchange is not clear. EF-Ts from *E. coli* has a C-terminal extension, which mimics the structure of the effector loop of EF-Tu (S. Thirup, personal communication). Why does it do that in the *E. coli* complex while this is not needed in the *T. thermophilus* complex?

The structure of the ternary complex of Phe-tRNA from yeast and EF-Tu:GDPNP from *T. aquaticus* [17] shows a very elongated complex, with the anticodon of Phe-tRNA pointing away from EF-Tu. The amino acid of Phe-tRNA is held in a pocket between domains 1 and 2 by hydrogen bonds to the main chain of the protein. The surface of domain 2 has a specific binding pocket for the terminal A-base of tRNA [18] while residues from all three domains recognize the 5′-phosphate. The backbone structure of the T-stem helix is bound to the surface of domain 3. The structures of both the active EF-Tu and the tRNA are very little altered during complex formation [17]. It has been shown recently that the crystal structure is similar to the structure found in solution [19].

The structure of the ternary complex of *E. coli* Cys-tRNA and *T. aquaticus* EF-Tu:GDPNP has very recently been determined [20]. The overall structure is very similar to that of the ternary complex with yeast Phe-tRNA [17], although the crystal packings are very different (Fig. 1). This structure determination confirms the basic assumption that structures of different tRNAs can efficiently be obtained in complex with EF-Tu:GDPNP. The Cys-tRNA is two nucleotides shorter than the canonical yeast Phe-tRNA, with deletions in the D-loop and in the variable loop. This tRNA structure displays some unusual features such as a unique G^{15}:G^{48} pair instead of the generally conserved 'Levitt pair', and such as two A-rich triples s^4U^8:A^{14}:A^{46} and A^9:A^{13}:A^{22}. The structure of EF-Tu:GDPNP in this complex is generally very similar to

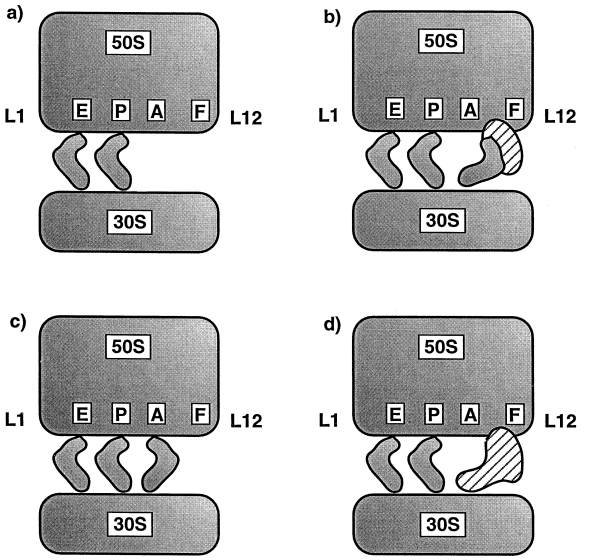

Fig. 2. A schematic representation of the binding states of tRNA and elongation factors to the ribosome. The view is from above and seen from the main protuberance side of the large subunit. The A, P, E-sites for the tRNA are indicated as well as the binding site for GTP hydrolyzing factors (F). a: tRNAs bound to the P- and E-sites. b: The binding of EF-Tu:GTP and tRNA. The factor binds to the classical factor binding site inside the L7/L12 stalk and the tRNA, while it remains bound to EF-Tu, binds to the decoding site of the 30S subunit. c: After GTP hydrolysis EF-Tu:GDP has dissociated and the tRNA has moved completely into the A-site. d: After peptidyl transfer EF-G binds to the ribosome and translocates the tRNAs and the mRNA. The figure illustrates the situation before EF-G:GDP dissociates from the ribosome.

the structure of the free factor. However, it is evident that side chains in contact with Cys-tRNA adapt to the local variations of the RNA structure.

The detailed mechanism of the GTP hydrolysis of EF-Tu has been a puzzle for some time. The main reason is that EF-Tu when compared to other G-proteins has a very low intrinsic GTPase activity [21]. EF-Tu works as a time-delayed molecular switch, and the GTPase activity is thus highly stimulated when the ternary complex interacts with the ribosome. Some information can be obtained by similarity with other G-proteins where structures with the transition state inhibitor AlF_3 have been obtained for the hetero-trimeric G-proteins [22,23] and for the ras-p21 protein [24]. Very elegant experiments strongly suggest that the γ-phosphate itself is activating a catalytic water [25]. The structural evidence indicates that an Arg residue is stabilizing the transition state. In the hetero-trimeric G-proteins this Arg is an internal residue found at the end of the effector loop, while for ras-p21, which does not have an Arg residue at this position, a similar stabilizing Arg is supplied from a GTPase activating protein [24].

In EF-Tu the situation is a little more complex. Arg^{59} of EF-Tu aligns with the internal residue in the hetero-trimeric G-proteins. However, mutations of this residue have very little effect on the GTP hydrolysis. Mutating Arg^{59} rather seems to influence binding of aa-tRNA [26,27]. Moreover, it has not been possible to use AlF_3 as a transition state inhibitor for EF-Tu (or EF-G) [28]. It is thus possible that the ribosome is providing a transition state stabilizing residue.

Crystals have recently been obtained of the quaternary complex of Phe-tRNA:kirromycin:EF-Tu:GDPNP [29]. A model for the overall structure has been determined, although at the present state of refinement a model for kirromycin cannot be unambiguously placed in density. However, residual densities are found in a cleft between domains 1 and 3 of EF-Tu as predicted from the positions of kirromycin resistant mutants [30]. The overall effect of the kirromycin is to bend domain 1 towards the tRNA. The structure of this quaternary complex is more closely similar to the density observed for the kirromycin inhibited ternary complex on the ribosome in the cryo-EM investigation [4].

4. Elongation factor G

The structure of EF-G is composed of four domains in addition to the G-domain. The structure of EF-G:GDP [31,32] from *T. thermophilus* can be accurately superimposed on that of the ternary complex of Phe-tRNA and EF-Tu:GDPNP [17]. Apart from an insertion in EF-G of a sub-domain in domain 1 and an insertion of a β-hairpin between domains 1 and 2, these domains have folds very similar to those of the corresponding domains in EF-Tu [33]. The rest of the structure of EF-G, domains 3, 4 and 5, mimics the tRNA of the ternary complex [34]. Since EF-G:GDP is the form that dissociates from the ribosome it is conceivable that EF-G:GDP leaves behind it an imprint on the ribosome suitable for the binding of the ternary complex [35].

In the case of EF-G the structure of the nucleotide free protein is very similar to the complex with GDP [32]. The structure of the complex with GTP remains difficult to crystallize. Thus we are left with indirect observations with regard to the structure of this complex and the conformational changes caused by GTP hydrolysis. One important piece of information in this regard is the locations of the mutations that lead to fusidic acid resistance for EF-G and their comparison to kirromycin resistance mutations in EF-Tu. These two antibiotics cause EF-G and EF-Tu respectively to stick to the ribosome after GTP hydrolysis. The large conformational change that causes EF-Tu to dissociate from the tRNA and the ribosome upon GTP cleavage leads to the loss of contact of helix C of the G-domain with the C-terminal domain 3. This general area is the binding site of kirromycin as described above. This is also the location of many of the kirromycin resistant mutations in EF-Tu [36]. It is possible that some of these prevent the binding of kirromycin, but it is also possible that some of them permit the conformational change despite the fact that kirromycin is bound. In the EF-G:GDP complex helix C of the G-domain also interacts with the C-terminal domain (domain 5). The binding site of fusidic acid is not known, but many fusidic acid resistant mutants are found in this area [37]. Thus it seems likely that the changes in domain contacts caused by GTP cleavage would involve the same face of the G-domain. However, one significant difference is that the macromolecular mimicry involves the ribosome binding complex of EF-Tu and the ribosome dissociating complex of EF-G.

5. The binding of elongation factors to the ribosome

A ternary complex of EF-Tu inhibited by kirromycin has been visualized on the surface of the ribosome using cryo-EM [4]. Comparison with a similar model of the ribosome with

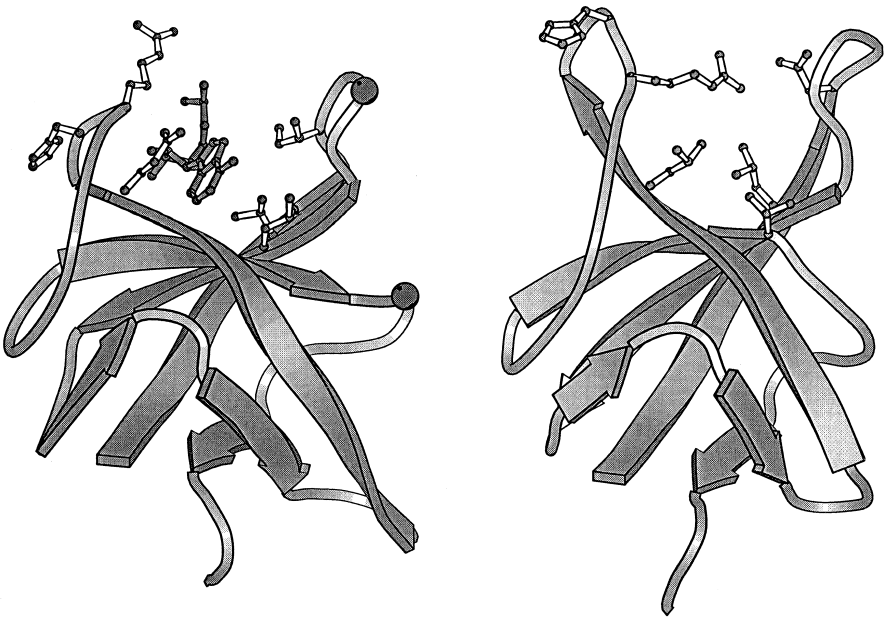

Fig. 3. Comparison of the structures of domains 2 from EF-Tu:GDPNP and EF-G:GDP. On the left side of the figure is EF-Tu:GDPNP with important residues in and close to the binding pocket for the terminal A-base. Residues are shown in ball-and-stick model, the A-base is in gray, and is seen end-on towards the phosphate and ribose. On the left side of the pocket are shown (from top to bottom): Arg^{274}, His^{273}, Glu^{271}. On the right side are: Ile^{231}, Val^{237}, Leu^{289}. All of these are conserved (or have conservative substitutions) and are involved in binding of the A-base. Notice that Arg^{274} makes a salt bridge to the phosphate of the nucleotide. Two larger balls indicate the positions of Gly^{233} (at the top) and Gly^{292}. When these glycines are mutated the interaction with the ribosome is impaired. On the right side of the figure is EF-G:GDP in a similar orientation, displaying a similar 'pocket'. On the left side are residues: His^{362}, Arg^{363}, Leu^{356}. On the right side: Asp^{319}, Leu^{325}, Val^{378}. Notice that the salt bridge of Arg^{363} and Asp^{319} fills the pocket. The picture is drawn with MOLSCRIPT [42].

tRNAs in the P and A sites shows clear difference densities in a shape that can be interpreted as the ternary complex. It also shows some local alteration of the position of the L7/L12 ribosomal protein, which is contacting EF-Tu near the GTP binding site, thus most likely inducing a rapid GTP hydrolysis. The structure of the ternary complex stalled on the ribosome in a codon-anticodon testing state, with the tRNA anticodon in the A site of the 30S subunit, indicates that the tRNA has to rotate so that the CCA-aa end can reach the peptidyl transfer center.

The main contacts between the ribosome and EF-Tu are between domain 1 and the 50S subunit and between domain 2 and the 30S subunit. This is interesting, because these two domains are common between EF-Tu and EF-G [33], but also because similar domains are present in all the translational G-proteins [38]. The implication is that all will bind to the ribosome in the same way, and that the GTPase activity will be stimulated by the same mechanism (Fig. 2).

One strange observation is that even the binding pocket for the terminal A-base found in EF-Tu is preserved in EF-G (Fig. 3). However, Glu^{271} of EF-Tu, which is important for the specific recognition of the A-base, is not conserved in EF-G. It is interesting that residue Gly^{233} in *T. aquaticus* EF-Tu (Gly^{222} in *E. coli*), which is found at the end of one of the two loops making up the pocket, when mutated to Asp is the so-called B_0 kirromycin resistant mutant of EF-Tu [39]. Although the B_0 mutant will allow kirromycin to bind to the ternary complex (forming a quaternary complex), this mutant has been shown to have impaired interaction with the ribosome [39]. It is thus quite possible that the ribosome by interacting with and modifying the local structure of this pocket helps release the aa-tRNA from the ternary complex. Also the EF-Tu mutant $Gly^{292}Val$ (Gly^{280} in *E. coli*), which is located at the same surface of domain 2, has lowered affinity for the ribosome [40].

Whether the GTP hydrolysis of EF-G precedes translocation of the peptidyl tRNA from the A-site to the P-site or not the EF-G:GDP complex is the dissociating form of the protein. Since this complex mimics the ternary complex of EF-Tu:GTP with tRNA it is likely to make an 'imprint' on the ribosome for the ternary complex [34,35]. The site it leaves will fit for the ternary complex. This leads to the prediction that EF-G:GDP will be bound to the ribosome very much like the kirromycin inhibited ternary complex as observed by cryo-EM. On the other hand, EF-G:GTP most likely will bind with its G-domain to the factor binding site below the L7/L12 stalk just like the ternary complex. However, the rest of the molecule will be in a conformation different from that of the ternary complex and thus not occupy any part of the A-site for tRNA. Translocation will then take place by a conformational change in EF-G so that its tRNA mimicking part will occupy the anticodon region of the A-site. Recent results [41] suggest that this conformational change and translocation is subsequent to the GTP hydrolysis.

References

[1] Liljas, A. and Al-Karadaghi, S. (1997) Nature Struct. Biol. 4, 767–771.
[2] Stark, H., Müller, F., Orlova, E.V., Schatz, M., Dube, P., Erdemir, T., Zemlin, F., Brimacombe, R. and Van Heel, M. (1995) Structure 3, 815–821.
[3] Agrawal, R.K., Penczek, P., Grassucci, R.A., Li, Y., Leith, A., Nierhaus, K.H. and Frank, J. (1996) Science 271, 1000–1002.
[4] Stark, H., Orlova, E.V., Rinke-Appel, J., Jünke, N., Mueller, F.,

Rodnina, M., Wintermeyer, W., Brimacombe, R. and van Heel, M. (1997) Cell 88, 19–28.
[5] Dube, P., Wieske, M., Stark, H., Schatz, M., Stahk, J., Zemlin, F., Lutsch, G. and van Heel, M. (1998) Structure 6, 389–399.
[6] Yonath, A. and Franceschi, F. (1993) Curr. Opin. Struct. Biol. 3, 45–49.
[7] Mueller, F. and Brimacombe, R. (1997) J. Mol. Biol. 271, 545–565.
[8] Mueller, F., Stark, H., van Heel, M., Rinke Appel, J. and Brimacombe, R. (1997) J. Mol. Biol. 271, 566–587.
[9] Mueller, F. and Brimacombe, R. (1997) J. Mol. Biol. 271, 524–544.
[10] Kaziro, Y. (1978) Biochim. Biophys. Acta 505, 95–127.
[11] Berchtold, H., Reshetnikova, L., Reiser, C.O.A., Schirmer, N.K., Sprinzl, M. and Hilgenfeld, R. (1993) Nature 365, 126–132.
[12] Kjeldgaard, M., Nissen, P., Thirup, S. and Nyborg, J. (1993) Structure 1, 35–50.
[13] Polekhina, G., Thirup, S., Kjeldgaard, M., Nissen, P., Lippmann, C. and Nyborg, J. (1996) Structure 4, 1141–1151.
[14] Abel, K., Yoder, M.D., Hilgenfeld, R. and Jurnak, F. (1996) Structure 4, 1153–1159.
[15] Kawashima, T., Berthet-Colominas, C., Wulff, M., Cusack, S. and Leberman, R. (1996) Nature 379, 511–518.
[16] Wang, Y., Jiang, Y., Meyering-Voss, M., Sprinzl, M. and Sigler, P.B. (1997) Nature Struct. Biol. 4, 650–656.
[17] Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B.F.C. and Nyborg, J. (1995) Science 270, 1464–1472.
[18] Kjeldgaard, M. and Nyborg, J. (1992) J. Mol. Biol. 223, 721–742.
[19] Bilgin, N., Ehrenberg, M., Ebel, C., Zaccai, G., Sayers, Z., Koch, M.H.J., Svergun, D., Barberato, C., Volkov, V., Nissen, P. and Nyborg, J. (1998) Biochemistry (in press).
[20] Nissen, P., Thirup, S., Kjeldgaard, M. and Nyborg, J. (1998) (in preparation).
[21] Wolf, H., Chinali, G. and Parmeggiani, A. (1977) Eur. J. Biochem. 75, 67–75.
[22] Sondek, J., Lambright, D.G., Noel, J.P., Hamm, H.E. and Sigler, P.B. (1994) Nature 372, 276–279.
[23] Coleman, D.E., Berghuis, A.M., Lee, E., Linder, M.E., Gilman, A.G. and Sprang, S.R. (1994) Science 265, 1405–1412.
[24] Scheffzek, K., Ahmadian, M.R., Kabsch, W., Wiesmüller, L., Lautwein, A., Schmitz, F. and Wittinghofer, A. (1997) Science 277, 333–338.
[25] Schweins, T., Geyer, M., Scheffzek, K., Warshel, A., Kalbitzer, H.R. and Wittinghofer, A. (1995) Nature Struct. Biol. 2, 36–44.
[26] Knudsen, C.R. and Clark, B.F.C. (1995) Protein Eng. 8, 1267–1273.
[27] Förster, C., Limmer, S., Zeidler, W. and Sprinzl, M. (1994) Proc. Natl. Acad. Sci. USA 91, 4254–4257.
[28] Kraal, B., de Graaf, J.M., Mesters, J.R., van Hoof, P.J., Jacquet, E. and Parmeggiani, A. (1990) Eur. J. Biochem. 192, 305–309.
[29] Kristensen, O., Reshetnikova, L., Nissen, P., Siboska, G., Thirup, S. and Nyborg, J. (1996) FEBS Lett. 399, 59–62.
[30] Abdulkarim, F., Ehrenberg, M. and Hughes, D. (1996) FEBS Lett. 382, 297–303.
[31] Czworkowski, J., Wang, J., Steitz, T.A. and Moore, P.B. (1994) EMBO J. 13, 3661–3668.
[32] Al-Karadaghi, S., Ævarsson, A., Garber, M., Zheltonosova, J. and Liljas, A. (1996) Structure 4, 555–565.
[33] Ævarsson, A., Brazhnikov, E., Garber, M., Zheltonosova, J., Chirgadze, Y., Al-Karadaghi, S., Svensson, L.A. and Liljas, A. (1994) EMBO J. 13, 3669–3677.
[34] Nyborg, J., Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Clark, B.F.C. and Reshetnikova, L. (1996) Trends Biochem. Sci. 21, 81–82.
[35] Liljas, A. (1996) Curr. Biol. 6, 247–249.
[36] Abdulkarim, F., Liljas, L. and Hughes, D. (1994) FEBS Lett. 352, 118–122.
[37] Johanson, U. and Hughes, D. (1994) Gene 143, 55–59.
[38] Ævarsson, A. (1995) J. Mol. Evol. 41, 1096–1104.
[39] Swart, G.W., Parmeggiani, A., Kraal, B. and Bosch, L. (1987) Biochemistry 26, 2047–2054.
[40] Tubulekas, I. and Hughes, D. (1993) J. Bacteriol. 175, 240–250.
[41] Rodnina, M.V., Savelsbergh, A., Katunin, V.I. and Wintermeyer, W. (1997) Nature 385, 37–41.
[42] Kraulis, P.J. (1991) J. Appl. Crystallogr. 24, 946–950.